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Research Article

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In vitro Anti-Coagulant Activity of Methanolic Extract of *Tradescantia spathacea*

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ABSTRACT

Hemostasis, a process of clots formation in the walls of damaged blood vessels to Necessity prevent abnormal bleeding and to regulate intravascular blood in liquid/fluid state of pharmacological and medicinal materials from plant source is going on increasing day by day due to its safety and less toxic properties. An investigational attempt from plants towards identifying and characterizing new anti-coagulants is worthwhile.

In this research we have expected to contemplate the conceivable anticoagulant impact of methanolic extract of Tradescantia spathacea by in vitro model utilizing blood tests of ordinary people. In vitro anticoagulant impact of Tradescantia spathacea in various focuses (5-20 mg/ml) was analyzed in the blood tests of typical people by estimating blood coagulating time, prothrombin time (PT) and aPTT (Activated fractional thromboplastin time). The concentrate was found to repress coagulation process and altogether delayed the coagulation time in a portion subordinate way. Further investigations can be done by in vivo models.

Keywords: Hemostasis; Clot; Anticoagulant; Methanolic extract; *Tradescantia spathacea;* Blood clotting time; Prothrombin time; aPTT (Activated Partial Thromboplastin Time)

INTRODUCTION

During periods of injury, Hemostasis acts as an interaction process between coagulation and anticoagulants that perpetuates the blood within the vascular system that got injured. It comprises a multiplex mechanism involving three major steps: (1) Vasoconstriction, (2) temporary blockage of a break by a platelet thrombus, and (3) coagulation of blood, or formation of a fibrin clot. The coagulation mechanism is a multiplex cascade mechanism indulging the formation of active enzymes from the precursor enzymes (zymogens, procoagulants, and proenzymes). Elements that are essential for coagulation are existed in an inert form which is converted to active when needed. Initially, one inactive enzyme is converted to its active form which converts the next inactive zymogen to its active enzyme. This continuous process proceeds until a fibrin clot is formed like a mesh. Membrane phospholipids surfaces, Protein factors and calcium ions play crucial role in the development of the fibrin clot [1].

To screen the extrinsic pathways and determine the deficiencies in Factors II, V, VII, and X, the Prothrombin Time (PT) test also known as pro-test or PT test is used. The extrinsic pathway in coagulation system gets activated by the thromboplastin in the presence of calcium ions and the subsequent clotting time depends on the concentration of blood clotting factors II, V, VII, and X. Hence, deficiency of one or more of these clotting factors (VII and X) indicates a prolonged PT and considered as abnormal [2-4]. Normal PT is 11-15 sec. Excluding nonsteroidal anti-inflammatory drugs like aspirin and indomethacin, some other important synthetic anticoagulant agents are heparin, warfarin, Ethylenediamine tetraacetic acid (EDTA) and citrate have anti-inflammatory and anti-platelets activity [5]. India is one of the nations sanctified with a rich heritage of traditional medicines and rich biodiversity to complement the herbal needs of the therapy done by these medicinal plants. India holds up recognize systems of medicines like Ayurveda Siddha and Unani which uses herbs and minerals in the formulations, but it is obligatory to evince traditional concepts scientifically in the laboratory [6]. The utilization of medicinal plants with widespread medicinal purposes for the treatment and prevention of various diseases is one of the most ancient traditional remedial forms of primary health culture [7]. Besides, the pharmacological properties, anticoagulant drugs show severe side effects. Hence, it is necessary to explore alternative anticoagulants having less side effects. Since the plants are the safe source of medicine, this study is an introductory attempt to investigate the *in vitro* anticoagulant activities of *Tradescantia spathacea* leaf extracts using standard experimental models in the blood samples of normal individuals.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Tradescantia spathacea* were collected from the garden of Vaageswari college of pharmacy, Ramakrishna colony, Thimmapur, Karimnagar District, Telangana in November 2019. The *T. spathacea* species were voucher specimen has been identified by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana, India. (BSI/DRC/2020/Identification/709).

Extraction of plant material

Tradescantia spathacea leaves were air dried at room temperature then crushed into powder using an electric grinder. This plant material was soaked by suspending 10 gms of powder in 100 ml of methanol with occasional stirring for 24 h. Later the suspension was filtered through a fine muslin cloth and then through a No.1 Whatmann filter paper. The solvent was eliminated at low temperature (40-50°C) under reduced pressure in a rotary evaporator to dryness.

Phytochemical analysis

Methanolic extract of the leaves of *Tradescantia spathacea* was subjected to a preliminary phytochemical analysis for the detection of phytochemical constituents present in the extract using different phytochemical tests.

Alkaloids

Dragendroff's reagent test: To 1 ml of extract, a few drops of Dragendroff's reagent was added to the test tube, and the color developed was noticed. The appearance of orange color shows the presence of alkaloids.

Saponins

Foam test: Take 1 ml of extract, add 10 ml of water and boiled. After few minutes, the mixture is shaken vigorously and filtered. The formation of froth and its persistence (1 cm height) for 1 h indicates the presence of saponins.

Steroids

Salkowski test: To the extract add 2 ml of chloroform, 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The change of color from red to blue and finally bluish indicates the presence of steroids.

Collection of blood and separation of plasma

10 ml of blood was drawn from healthy volunteers (with no medicine consumption history through vein puncture. 3.8% trisodium citrate solution (1 part of trisodium citrate solution: 9 parts of blood) was added to prevent natural coagulation process. Immediately centrifugation was done for 15 min at 3000 xg to obtain pure platelet plasma (PPP). The plasma was separated and refrigerated at -4°C for further use.

Blood clotting time measurement

In vitro blood coagulating time estimation was done utilizing a strategy for Lee and White as announced by Osoniyi et al. [8] doing a few alterations [9]. Cylinders containing 0.5 ml every one of rough concentrate and divisions of *Tradescantia spathacea* suspended in Phosphate Buffered Saline (PBS) at different centralizations of around 5-20 mg/ml, Acid citrate dextrose (anticoagulant) and PBS (control), were brooded in a water shower at 37°C. Newly drawn blood (0.5 ml) was deliberately moved into the substance of every one of the brooded tubes, while at the same time beginning a stopwatch [3]. After 30 sec. stretch, the cylinders were inclined at an edge of 45° to check for the coagulation arrangement. The ideal opportunity for the underlying perception of clump was noted, and the inclining proceeded at appropriate stretches until the cylinders could be transformed without streaming of blood. The stopwatch was halted, and the time was noted as the last coagulating time. (Tables 1 and 2 and Figure 1).

Prothrombin Time (PT)

Prothrombin Time (PT) was resolved by the strategy for Brown [9], as announced by Osoniyi et al. [8]. Calcium Thromboplastin Reagent unit was utilized. Calcium Thromboplastin reagent was reconstituted [3] with refined water and pre-warmed in a water shower at 37°C for around 10 mins. Blood plasma (0.1 ml) was pipetted into coagulating tubes and was hatched in a water shower for 2-3 mins at 37°C. At that point, 0.1 ml of test separate at different centralizations of 5, 10, and 20 mg/ml were included and PBS (for control). 0.1 ml of Calcium thromboplastin reagent was promptly added to the blend, while all the while starting a stopwatch. The cylinders were delicately inclined at 45° edge at ordinary spans until a coagulation was shaped. The stopwatch was halted promptly, and the coagulation arrangement time was recorded (Table 3; Figures 2 and 3).

Activated partial thromboplastin time (aPTT)/Kaolin Cephalin Clotting Time (KCCT) aPTT or KCCT was resolved by the strategy utilized by Brown, as announced by Osoniyi et al. [8] furthermore, utilizing a Diagen Kaolin reagent pack [3]. The halfway thromboplastin reagent (Kaolin Platelet substitute blend [3]), was reconstituted by the makers guidance. The coming about suspension and 0.02 M Calcium chloride were pre-warmed at 37°C in a water shower independently [3]. 0.1 ml Plasma was pipetted into thickening cylinders and brooded in a water shower for 2-3 mins at 37°C, at that point 0.1 ml of halfway thromboplastin reagent was added to the cylinders and the substance were quickly blended. The blend was additionally hatched for 3 mins, after which separate arrangement at different groupings of 5, 10, and 20 mg/ml were included, 0.1 ml of PBS (for control), followed by 0.1 ml of pre-warmed

Calcium chloride, while at the same time beginning a stopwatch. The cylinders were inclined at normal stretches until a coagulation is framed. The stopwatch was stopped immediately, and the clot formation time was recorded. (Table 4 and Figure 3).

RESULTS

Phytochemical analysis

Preliminary phytochemical analysis was performed and steroids, glycosides, alkaloids, tannins, saponins and flavonoids were identified.

Table 1. Phytochemical analysis.

S.NO	Tests	Methanolic extract
1	**STEROIDS**	
	Salkowski test	+
2	**GLYCOSIDES**	
	Baljet test	+
4	**ALKALOIDS**	
	Dragendroff's test	+
5	**TANNINS**	
	Lead acetate test	+
	Bromine water	+
6	**SAPONIN GLYCOSIDES**	
	Foam test	+
	Haemolytic test	+
7	**FLAVONOIDS**	
	Schinoda test	+

Table 2. Blood clotting time.

Concentration	Blood clotting time(min)
5 mg/ml	5.24
10 mg/ml	10.87
20 smg/ml	18.24
control	0.81

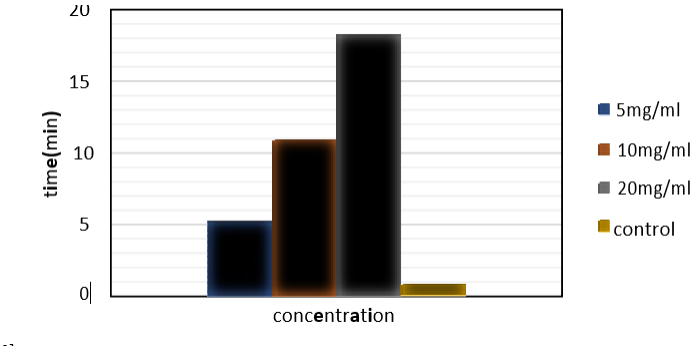

Figure 1. Showing blood clotting time of methanolic extract of *T. spathacea* at various concentrations (5-20 mg/ml).

Table 3. Prothrombin time.

Concentration	Time(sec)
control	20
5 mg/ml	40.35
10 mg/ml	65.74
20 mg/ml	80.78

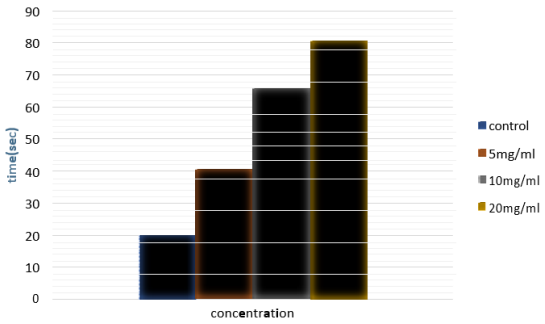

Figure 2. Showing prothrombin time of methanolic extract of *T. spathacea* at various Concentrations (5-20 mg/ml).

Table 4. Activated partial thromboplastin time (aPTT)/ Kaolin cephalin clotting time (KCCT)

Concentration	Time(sec)
control	35.2
5 mg/ml	54.35
10 mg/ml	76.5
20 mg/ml	85.92

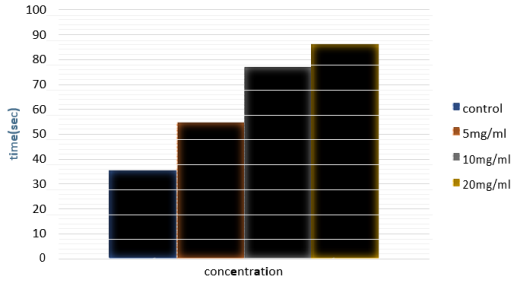

Figure 3. showing aPTT of methanolic extract of *T. spathacea* at various concentrations (5-20 mg/ml).

DISCUSSION

Recent years, chemical substances being derived from natural plant sources are attracting the interest of researchers and are being tested for their anti-coagulant properties, as a new molecule for the development of new drugs. Plants do have variety of chemical constituents that are used to perform biological functions. These phytochemicals can be

safely introduced to humans with long term health benefits and less toxicities. *Tradescantia spathacea*, in our study has shown the anti-coagulant property which can be further analyzed by *in vivo* models and the chemical constituents responsible particularly can be isolated and used in further studies.

CONCLUSION

It can be suggested that the methanolic extract of *Tradescantia spathacea* has the anticoagulant property which can be exploited in the treatment of blood coagulation disorders. Further *in vivo* studies should be performed to evaluate the mechanisms of the constituents responsible for the anti-coagulant property.

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